

## Temperature Effects on Otolith Microstructure and Birth Month Estimation from Otolith Increment Patterns in Atlantic Menhaden

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**Abstract.**—We demonstrate temperature-influenced increment spacing in the sagittal otoliths of juvenile Atlantic menhaden *Brevoortia tyrannus* reared at different temperatures and describe otolith increment patterns from wild juveniles born in different months. Tank-reared individuals completed metamorphosis a month earlier at 25°C than at ambient temperatures (10–14°C). We identified several patterns from the sectioned otoliths, including reduced time to metamorphosis, faster transition to the juvenile stage, and increased increment spacing after onset of metamorphosis for those individuals exposed to warmer temperatures. For wild individuals that could be accurately aged, mean otolith increment spacing in the zone from 70 to 80 increments increased 1–2 µm for fish born in November and December to more than 3 µm for fish born in February. The increase in increment spacing is consistent with the arrival of individuals across seasonally warming temperatures in the estuary. A discriminant analysis based on otolith increment spacing and metamorphic patterns successfully classified birth month for 59–100% (65% overall) of Atlantic menhaden spawned between November–February. This approach is being developed ultimately to identify the birth month of wild Atlantic menhaden adults in order to test for differential survival among cohorts born in different months that recruit to the fishery.

A primary goal of fisheries research is to identify factors that affect recruitment (Fritz et al. 1990; Laurence 1990). By using an otolith-based analysis of survivors and comparing distribution of birth dates across various stages several fisheries recruitment studies have identified the life history stage crucial to the determination of year-class strength (Methot 1983; Crecco and Savoy 1985; Rice et al. 1987; Moksness and Fossum 1991). Identification of the stage that is most important for year-class determination (e.g., egg, larval, juvenile, late juvenile) allows a better focus on the particular mechanisms critical to recruitment success.

For Atlantic menhaden *Brevoortia tyrannus* in North Carolina waters, annual and seasonal recruitment patterns based on birth dates (deter-

mined from late-stage larvae) necessary for this type of survival analysis are well established (Warlen 1994). Our study was directed at determining how much information pertaining to events during early life history can be extracted from otoliths of older Atlantic menhaden that have survived the juvenile stage and recruited to the fishery. If our resolution of otolith information is fine enough to distinguish birth date cohorts, a comparison of birth date or growth-rate distributions from these older fish and earlier ages (e.g., larvae and early juveniles) could reveal selective survival that may have occurred during the juvenile stage. The importance of focusing this type of analysis on adults hinges on the finding that age-1 and older fish can be sampled at the population level—being a time when they are highly vulnerable to the commercial fishery—whereas late juveniles are widely dispersed and difficult to sample adequately (Chester and Waters 1985; Ahrenholz et al. 1989).

Samples of adult Atlantic menhaden from the commercial fishery are readily available through an established survey program (see Chester and

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Waters 1985; Smith 1991), but we cannot determine daily ages of these older fish. Good confidence in daily aging ( $\pm$  about 7 d) can only be achieved for juveniles up to about 200 d of age (Ahrenholz et al. 1994). Alternatively, we may be able to use a discriminant analysis approach to determine birth dates of older fish based on otolith increment width patterns established during their early life history. Preliminary work has strongly suggested that increment spacing patterns are related to water temperature at the time of arrival of larvae in estuarine nursery areas. Temperature is known to affect increment spacing in otoliths of many species (Taubert and Coble 1977; Neilson and Geen 1982; Campana 1984; Mosegaard et al. 1988; Wright 1991; Bradford and Geen 1992; Francis et al. 1992). Therefore, if strong temperature gradients are experienced in the early life of a fish, the effects of those temperatures may be detectable in the otolith increment pattern.

Atlantic menhaden experience a temperature gradient between the spawning grounds as eggs and the nursery habitat as juveniles. Spawning is thought to occur primarily at temperatures of 18°C and above (Warlen 1994; Fitzhugh and Hettler 1995), eggs and early larval stages have been found between the 15°C and 20°C isotherms in the mid-Atlantic Bight (Kendall and Reintjes 1974), and juveniles colonize the estuary over a broad seasonal temperature range from less than 10° to mid-20°C during any given year (Miller et al. 1986; Warlen 1994). Therefore, early otolith increment spacing patterns, particularly during the larval to juvenile transition, may be useful for determining birth dates with seasonal precision (i.e., distinguishing fall-, winter- and spring-spawned individuals from the birth modes commonly observed in North Carolina; see Warlen 1992, 1994). A similar approach has been used to distinguish spring-spawned from autumn-spawned Atlantic herring *Clupea harengus* in the North Sea (Moksness and Fossum 1991).

Because somatic metamorphosis in Atlantic menhaden is fairly pronounced when viewed morphologically (Lewis et al. 1972; Warlen 1992), metamorphic marks or features of the otoliths may also be useful as a benchmark of seasonal change. Transition areas in otoliths are associated with changes in ontogeny, such as settlement and metamorphosis, and these areas are often used as reference points for measuring larval duration and premetamorphic and postmetamorphic growth (Brothers and McFarland 1981; Victor 1982; Francis et al. 1992; May and Jenkins 1992; Toole et

al. 1993). If these features can be recognized from otoliths and they can be associated with seasonal changes, then they may also be useful for estimating birth dates.

Our objectives were to describe temperature-influenced patterns in increment spacing and metamorphosis from the otoliths of juvenile Atlantic menhaden reared in a controlled-temperature trial, to relate these descriptions to otolith patterns from wild juveniles young enough to be accurately aged, and to thereby test the usefulness of an otolith-based pattern analysis that could be used to assign seasonal birth dates to adults. This process will eventually allow us to extend our analysis of survivors to the adult stage.

### Methods

Larval Atlantic menhaden were collected from 28 January to 4 February 1994, with a neuston net at Pivers Island, Beaufort, North Carolina. All larvae were acclimated to ambient temperatures (10–12°C) and salinities (about 32‰) until 7 February; they were then immersed for 15 h in a solution of 100 mg alizarin complexone (ALC)/L. This method incorporated a fluorescent mark on the sagittae of each larva as a time reference point following procedures reported earlier (Ahrenholz et al. 1994). Groups of about 160 larvae were sorted into each of four 1,000-L treatment tanks. In three of these tanks, temperatures were adjusted upwards over the next 48 h to approximately 15, 20, and 25°C and then held relatively constant either until the end of the experiment or until increasing ambient temperatures surpassed the treatment temperature (Figure 1). Because the tanks were located in a greenhouse, larvae were subjected to a natural photoperiod. In all treatments, larvae were fed the same dry rations (Ziegler salmon starter) from 24-h automated feeders that provided continuous food delivery. We maintained a feeding level that was just to excess for the ambient and 15°C groups. To maintain this level, rations for each tank started at 17 mL/d and were increased by approximately 2–3 mL each week until a ration of 40 mL/d was reached on 9 May. Rations were then held constant for the duration of the experiment. To compare rates of metamorphosis, about 20 fish were sampled from each tank beginning 1 month after ALC marking (10 March) and every 2 weeks thereafter until the last sampling date on 16 May (Figure 1). However, fewer numbers of fish were available for sampling on the last two dates (Figure 1 and see below).

Wild juvenile Atlantic menhaden were sampled

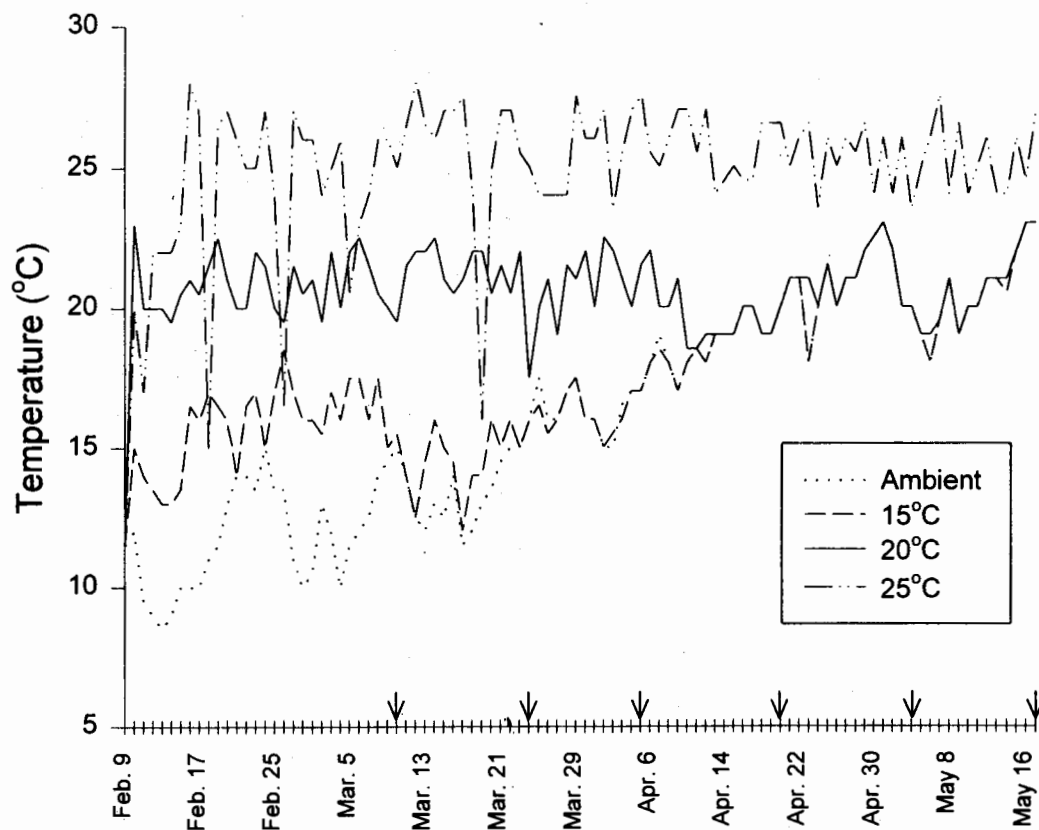


FIGURE 1.—Daily morning temperatures for the experimental rearing tanks. Alizarin complexone marking of Atlantic menhaden otoliths was completed on 8 February. Arrows along the x-axis indicate sampling dates.

approximately every 2 weeks from 20 April to 6 July 1994 from two North Carolina nursery creeks: Hancock Creek, a tributary of the Neuse River, and South Creek, which flows into the Pamlico River. Samples were collected randomly with a cast net in April and May; by late May, we conducted night sampling stratified along stream lengths, using a  $1.5 \times 1.5$ -m push net (mesh size = 9.5-mm-bar body, 6.4-mm-bar cod end). This gear is most efficient for sampling juvenile clupeids of 35–70 mm total length, TL (Kriete and Loesch 1980; Dixon and Loesch 1992); thus, we retained all juveniles captured within this size range. From our collections, we extracted otoliths from 10 randomly subsampled juveniles taken each month (April–July) in order to insure that our samples reflected a range of birth dates, allowing us to examine seasonal increment patterns.

Upon sampling, Atlantic menhaden from both the field and laboratory trials were placed on ice until standard lengths (nearest 0.5 mm) and weights (nearest 0.1 mg) were measured and sagittal otoliths removed (within 24 h). For the temperature treatments, sagittal otoliths were random-

ly selected from the remaining available fish from the last two sampling dates (about 10 from each treatment) to compare the increment patterns recorded over the duration of the experiment. For both wild and temperature treatment otoliths, we prepared sections as previously described (Ahrenholz et al. 1994). Briefly, right or left sagittae were randomly selected, sectioned transversely, and ground and polished to the primordia on both sides. For otolith terminology and increment interpretation we followed Campana (1992). We made counts along a curvilinear axis of each otolith section to the first and second transition marks (defined below, see Figures 2, 3) and ALC marks. Measurements along this axis were also taken for every successive 10-increment count beginning with first feeding (see Maillet and Checkley 1990) to determine the average increment width across the 10 increments. Although the resolving limit of our microscope (at  $1,200\times$ , numerical aperture 1.3) is about  $0.2 \mu\text{m}$ , we rounded measurements made on an image analysis system to the nearest  $0.1 \mu\text{m}$  for calculations. We were careful to reduce bias in increment measurements by polishing sec-

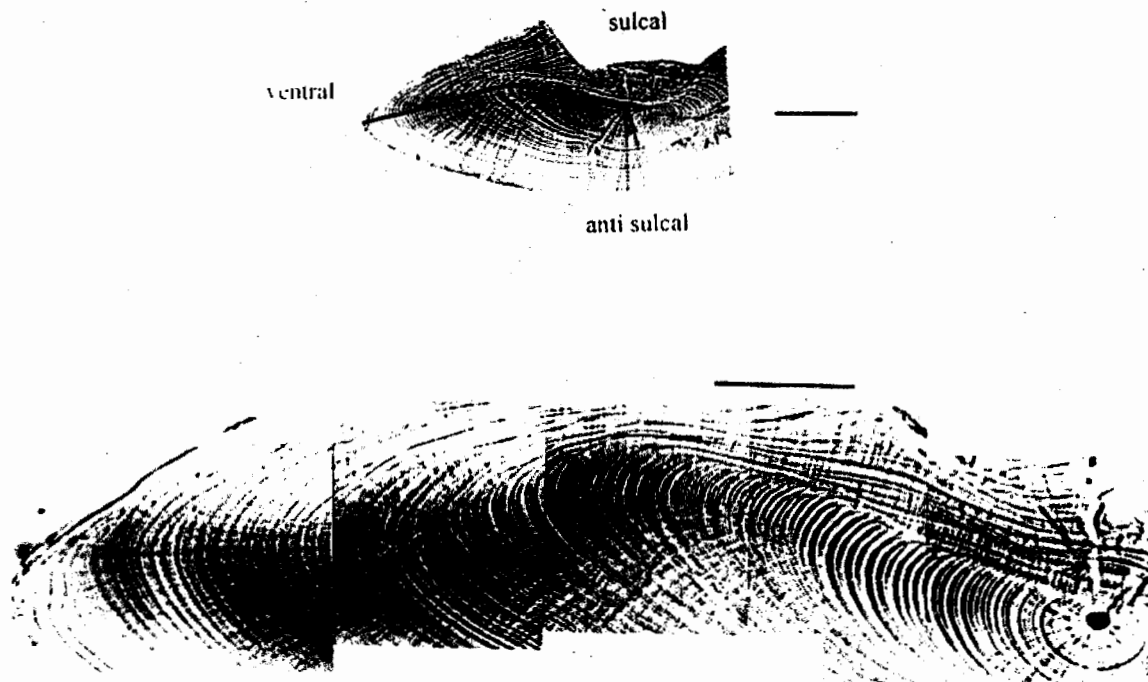


FIGURE 2.—A cross-sectioned sagittal otolith from a 1.07-g (38.9-mm standard length) juvenile Atlantic menhaden captured on 21 April 1994 and estimated to have hatched on 8 November 1993 (estimated age = 160 increment counts + 5 d to first feeding = 165 d). In the bottom panel, the prejuvenile zone is bounded by the first and second transition marks, denoted by arrowheads (scale bar = 50  $\mu\text{m}$ ). The top panel shows the axis of measurement (scale bar = 100  $\mu\text{m}$ ).

tions as thin as possible ( $<20 \mu\text{m}$  thickness) to minimize focal plane variation affecting the appearance of increment widths, by keeping the same focal plane across each set of successive 10-increment widths, and by consistently measuring along the axis of growth for each otolith (see Campana 1992). Because changes in the axis of otolith growth can affect increment spacing, which in turn may depend on the orientation of the sections viewed, caution in interpretation of increment widths is necessary (Campana 1992). We used the ventral rather than the dorsal axis of cross sections for measurements because this orientation revealed a greater change in the axis of growth, presumably associated with metamorphosis, and facilitated our measurement of where these changes occurred. The dorsal side of the sections, which we did not use in this analysis, usually revealed a straight measurement axis that bisected the widest area of the otolith increments and probably would be a more preferable orientation if we were back-calculating fish length based on otolith size. We followed Lewis et al. (1972) to define the ontogenetic stage of Atlantic menhaden relative to their transformation from the larval to prejuvenile

to juvenile stages based on changes in weight and length.

The appearance of metamorphosis within fish otoliths has been variously described. We used several features, which appear to be common, to aid us in identifying the pattern in Atlantic menhaden. Increments associated with metamorphic transition become compressed near the end of the planktonic stage and are often bounded by broad, dark, and more widely spaced increments that are more clearly associated with either the larval or juvenile stage (Victor 1986; Francis et al. 1992). This increment compression is often abrupt in tropical fishes (hence the terms "metamorphic" or "settlement" mark), but larval increments have been noted to become reduced in width toward the onset of settlement or transition to a juvenile phase (Victor 1986; Pitcher 1988; Thorrold and Milicich 1990). Because metamorphosis is not abrupt in Atlantic menhaden, either viewed somatically or from apparent features in the otolith, we refer to the metamorphic pattern as a "zone" bounded by first and second transition marks (see Results).

We conducted a discriminant analysis of the wild juvenile otoliths to test our ability to predict

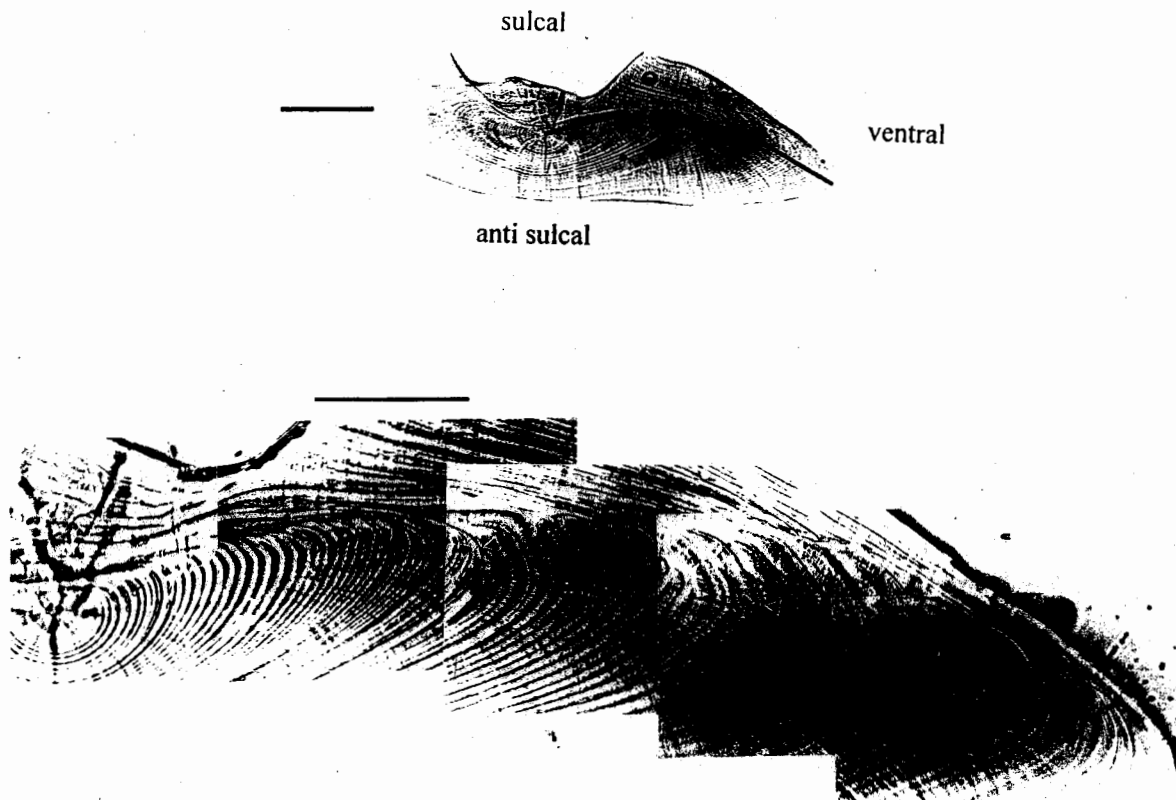


FIGURE 3.—A cross-sectioned sagittal otolith from a 0.79-g (35.6-mm standard length) juvenile Atlantic menhaden captured on 31 May 1994 and estimated to have been hatched on 22 January 1994 (estimated age = 125 increment counts + 5 d to first feeding = 130 d). In the bottom panel, the prejuvenile zone is bounded by the first and second transition marks, denoted by arrowheads (scale bar = 50  $\mu\text{m}$ ). The top panel shows the axis of measurement, (scale bar = 100  $\mu\text{m}$ ).

birth dates based on increment count and spacing variables. After aging our samples of juveniles from the 1993–1994 year-class, we assigned individuals to their month of birth (November, December, January, or February). Our next procedure was to order and select the variables with the most predictive and discriminatory power from all the count and measurement variables described earlier by using an ad hoc forward-selection approach described by Huberty (1984). Briefly, all the variables were tested singularly with the Proc Discrim procedure (SAS Institute 1988) to determine the number of correct classifications to month of birth. Next, multiple-variable subsets (starting with the most predictive pairs of variables) were sequentially tested until the variable subset with the highest number of correct classifications was determined. After reducing the number of possible discriminatory variables, we used a linear rule to derive the discriminant function (Huberty 1984; SAS Institute 1988). Finally, we used a cross-validation procedure to generate a classification table of ac-

tual and predicted birth date groupings because this bootstrapping method minimizes bias in the error-count estimate (Huberty 1984; SAS Institute 1988).

### Results

We detected varied increment spacing patterns from preliminary observations of otoliths from wild juveniles with different seasonal birth dates. Our first objective was to characterize these patterns based on our confidence in aging otoliths from increment counts (see Ahrenholz et al. 1994). For example, Figure 2 shows the typical pattern from a cross-sectioned sagitta of a fall-spawned Atlantic menhaden collected in early April. Initial increment widths of the early larval stage widen from the primordium along the axis of measurement and then reduce down in width to the point where the otolith is rapidly changing its axis of growth when viewed in cross section (first set of arrows). This first transition point shows features associated with the onset of metamorphosis in

some fishes. The reduction in width of larval increments reaches a point where there is an abrupt change in the appearance of the increments from continuous to a discontinuous or overlapping pattern, possibly due to a reduction in growth of the otolith in the sulcal-antisulcal direction (see Francis et al. 1992). This point (about 89 increment counts; estimated age 94 d) corresponds to a standard length (SL) of about 25 mm (Warlen 1992) and total length = 30 mm,<sup>2</sup> which is equivalent to the size at transition from the larval to the pre-juvenile stage (Lewis et al. 1972). Based on its estimated birth date and the typical ages of larvae arriving at inlets across a 4–6 month period, we expected that this individual colonized the estuary in December after a larval at-sea duration of about 40–50 d (Warlen 1994), and by these estimates, metamorphosis would have begun after entering the estuary. The set of arrows furthest from the primordium indicates the transition to a more regularly spaced pattern of broad increments (along the ventral axis of measurement) typical of juvenile growth. At this point, increments are again continuous (although compressed) in the sulcal and antisulcal directions (Figure 2).

By comparison, an individual with a much later estimated birth date of 22 January (which probably colonized the estuary at a later and warmer time—February or March—see Warlen 1994) exhibits a much abbreviated zone in which increments are reduced in width. As a consequence of an abbreviated zone of reduced increment widths, the first transition in the axis of otolith growth is seen at about 52 increment counts (estimated age about 57 d; Figure 3). But as the recruitment season progresses, Atlantic menhaden larvae arriving at Beaufort inlet are larger and older, and typical at-sea durations for fish arriving at Beaufort Inlet in February or March, usually 70–80 d, are longer than for fish arriving in fall and winter (Warlen 1994). This contrast suggests that metamorphosis may be underway for winter-spawned fish before they reach the estuary. Also, the increment spacing is visibly wider in the area that should represent prejuvenile growth based on increment counts (area between arrows, Figure 3). These differences in increment patterns associated with different birth dates were consistent among fish and suggested an effect of temperature on increment pattern, prompting us to examine metamorphosis and

otolith increment widths as a function of temperature under controlled rearing conditions.

By our first sampling period for the rearing experiment (10 March 1994), 93% of the Atlantic menhaden had completed the early phase of metamorphosis from larvae to prejuveniles (>70 mg), according to the size criteria of Lewis et al. (1972). We observed the later phase of metamorphosis from prejuveniles to juveniles at a weight of about 469 mg (Figure 4), which agrees with earlier results of Lewis et al. (1972). But the transformation rates varied among treatments. Atlantic menhaden from the two warmest treatments completed metamorphosis 1 month into the experiment (10 March), while some fish at ambient and 15°C were still metamorphosing on 5 April (Figure 4). Differences in completing transition to the juvenile stage varied up to about a month among our treatments, prompting us to determine whether these temperature effects were recorded in the otoliths. We examined otoliths of fish taken on the last two sample dates of the trial (4 May and 17 May), at which time all fish had visibly completed transition to the juvenile stage.

All the fish used in the rearing trial showed similar increment spacing patterns for the at-sea larval stage and the reduced increment width zone (Figure 5), which we expected because the fish arrived at the inlet and were collected at about the same time in late January. Consequently, there was little difference in the mean number of counts to the first transition zone (range in mean count, 54–61), which we expected because of the similar size and collection times of late stage larvae (Figure 5). The mean counts to the ALC marks ranged from 72 to 80 among the treatments. This interval indicated that there were small differences in age when the fish were marked or that there were possible variations in counts to the ALC mark where the effects of the temperature treatments should first be recorded in the otolith.

Treatment effects were most evident both in the counts to the second transition mark and in the increment spacing in the zone between the first and second transition marks. Increment counts to the second transition mark decreased with increasing temperature, which supports our visual observations of faster metamorphic transitions (in somatic appearance and weight) with increased temperature (Figure 5). At the warmest temperatures, mean increment counts between the ALC and the second transition marks were 36 and 26, respectively, for the 20 and 25°C groups (Figure 5); completion of somatic metamorphosis was noted 31 d

<sup>2</sup> Total length = 1.4 SL - 5.2;  $r^2 = 0.99$ ,  $N = 79$ , range = 14.4–31.2 mm SL.

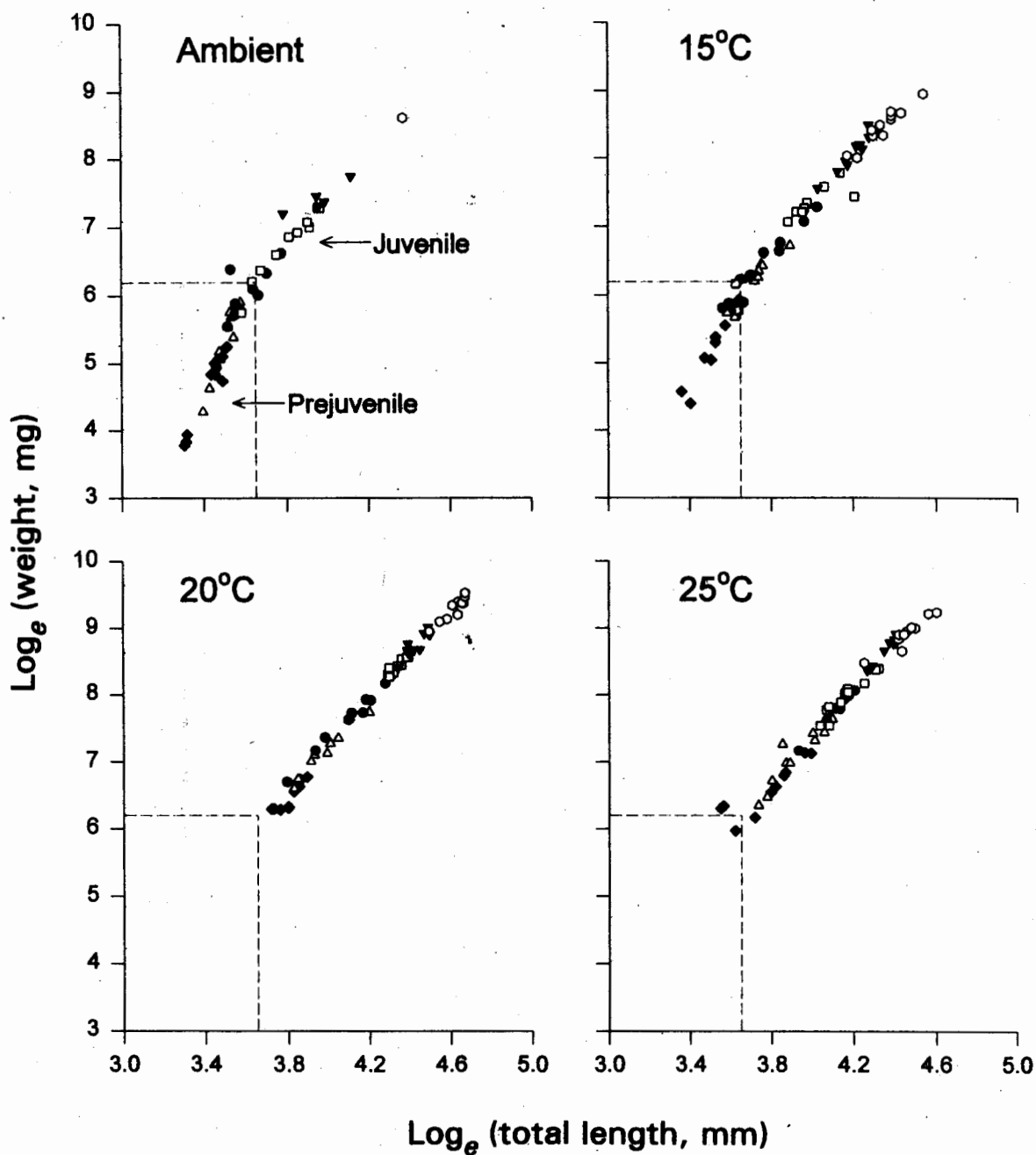


FIGURE 4.—Length-weight relationships for Atlantic menhaden sampled about every 2 weeks from the temperature rearing trial. The bisecting lines indicate the inflection point between the prejuvenile and juvenile growth stages (based on Lewis et al. 1972).

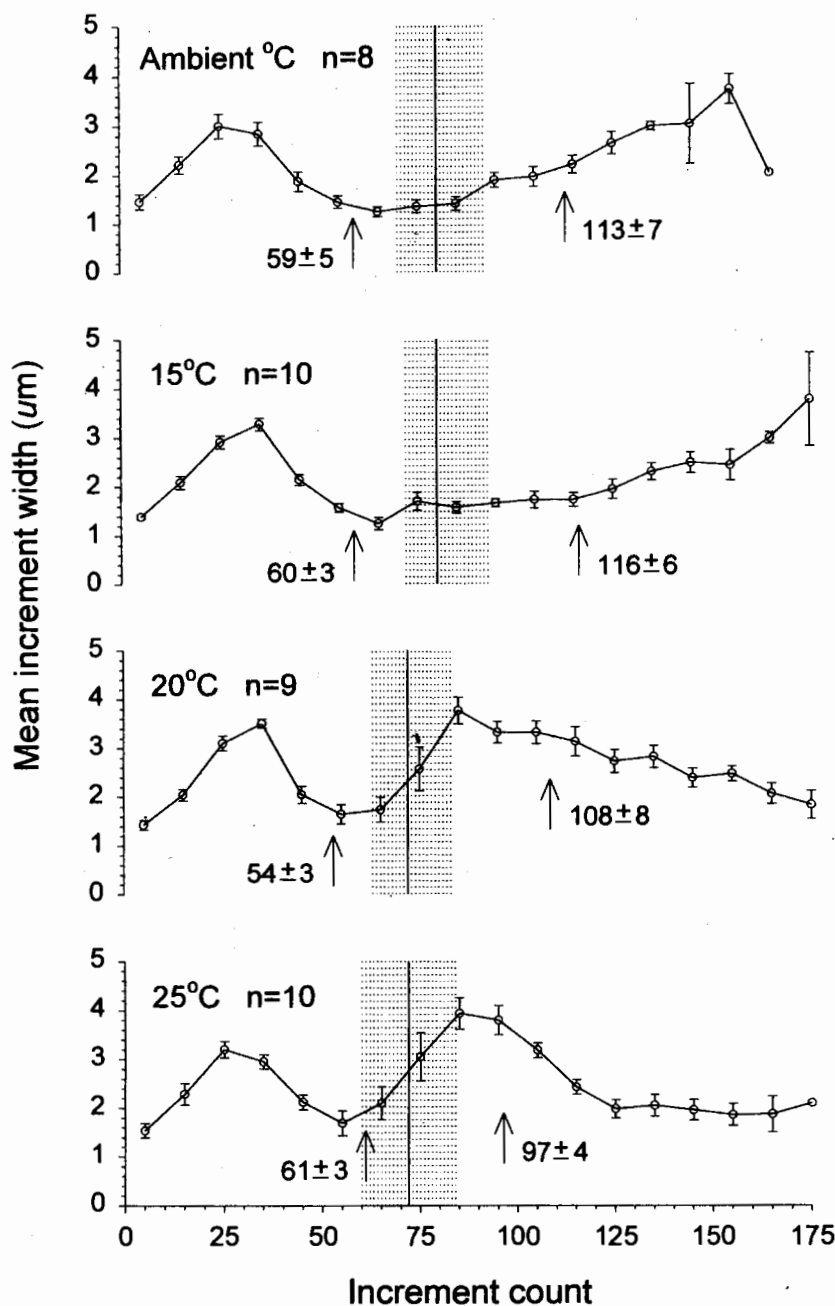


FIGURE 5.—Average increment widths ( $\pm$ SE) for each successive 10 increments from first feeding to the vertical edge of the sagittal cross sections of otoliths of Atlantic menhaden from the controlled temperature trials. The initial increase and then decline in increment width corresponds to the at-sea larval stage, which occurred prior to capture. The shaded zone and vertical lines indicate the range and mean increment count to the alizarin complexone mark for each treatment group. The arrows and associated numbers indicate the mean ( $\pm$ SE) increment count (among fish examined) of the transition marks that correspond to changes in the axis of otolith growth observed in cross sections.

after marking on our first sampling date (10 March, Figure 4). For the coldest treatment, increment counts to the second transition mark did not agree as closely with the estimated time to somatic meta-

morphosis. The difference in mean increment counts between the ALC and the second transition marks was 33, and the estimated time to complete somatic metamorphosis was about 57 d after mark-



ing (5 April; Figure 4). Although increment counts to the second transition mark may have only been suggestive of treatment effects, a prominent treatment effect on increment widths occurred after the onset of temperature treatments denoted by the ALC mark. Increment widths increased with increasing temperature in a zone between about increment count 70 and count 95 (Figure 5). In addition, increment widths decreased with increasing temperature in the zone between counts 150 and 160 (Figure 5).

To see if metamorphic-temperature patterns were evident in the otoliths from random samples of wild juveniles, we graphed the increment width measurements grouped by birth months (November, December, January, and February; Figure 6). The November and December groups, which we expected to be subjected to the coldest temperatures during metamorphosis, exhibited a similar pattern to the cold temperature treatments (ambient and 15°C); increments near the first transition zone were just greater than 1  $\mu\text{m}$  in mean width. These groups also showed a relatively long period or large zone containing reduced increment widths. By comparison, the reduced increment width patterns were shorter in duration for fish with January and February birth dates, and mean increment widths at the first transition only decreased to about 2  $\mu\text{m}$  or greater. Mean counts to both the first and second transition zones decreased for later birth dates, and differences were apparent in spacing for the transition zone. At 70–80 increment counts, mean widths were 1–2  $\mu\text{m}$  for the November and December birth dates but increased to greater than 2  $\mu\text{m}$  for January birth dates and to greater than 3  $\mu\text{m}$  for February birth dates (Figure 6). These results are consistent with arrival of individuals across seasonally warming temperatures in the estuary.

To examine this pattern within wild juveniles further, we plotted a linear regression of the estimated date that increment 75 was formed (based on back-calculated birth date) versus mean increment width within the band from 70–80 increments. The regression indicated that increment width within this zone of 70–80 increments was a fair predictor of increment formation date for those juveniles of estimable age ( $r^2 = 0.57$ ; Figure 7). For early dates for which increment spacing was on the order of 1–2  $\mu\text{m}$ , it was apparent that individuals were late larvae or just transforming to prejuveniles. For later dates for which mean increment spacing increased to greater than 2  $\mu\text{m}$ , these increment counts were beyond the zone

where the cross-sectioned axis of otolith growth changes, which is consistent with the onset of metamorphosis and beginning of the prejuvenile stage.

To increase our ability to predict birth date from wild juveniles, we used a discriminant analysis that began with an ad hoc forward-selection procedure. We retained five variables with the greatest predictive power (Table 1). The mean increment width between counts 70 and 80 and the mean width between counts 90 and 100 had the greatest predictive power for single variables (Table 1), which supported our previous expectations about which variables would be most predictive based on increment-width graphs (Figures 6, 7). Ten of the original 15 variables were eliminated, including measurements for successive increment groups to increment 50, mean widths for group 80–90, and count to the first transition mark (Table 1). No widths for increments beyond count 130 were used in the analysis because we wanted to include the youngest individuals (spawned in February) for developing the discriminant function.

Results of the cross-validation procedure show that a discriminant function based on otolith increment characteristics offers substantial capability to classify Atlantic menhaden to birth month. Members of the November group were clearly separated from later birth date groups with no misclassifications (Table 2). The December group showed only three misclassifications, which was the second highest correct classification rate. Members from the January and February groups were more commonly confused and had correct classification rates of 59 and 60%, respectively. No members of any group were misclassified by more than a month except for a single individual in the February birth group.

### Discussion

An otolith-based approach has been used to identify spawning cohorts of fishes based on the earliest larval increment patterns and changes in temperatures on the spawning grounds (Moksness and Fossum 1991; May and Jenkins 1992). In the mid-Atlantic Bight system however, temperature in the early larval environment is relatively constant throughout the spawning season as a result of the migratory pattern of adult Atlantic menhaden and the proximity of spawning areas to the Gulf Stream, but inshore and nursery temperatures change dramatically (Stefansson et al. 1971; Warlen 1992, 1994). Therefore, we hypothesized that seasonal temperature signatures in increment pat-

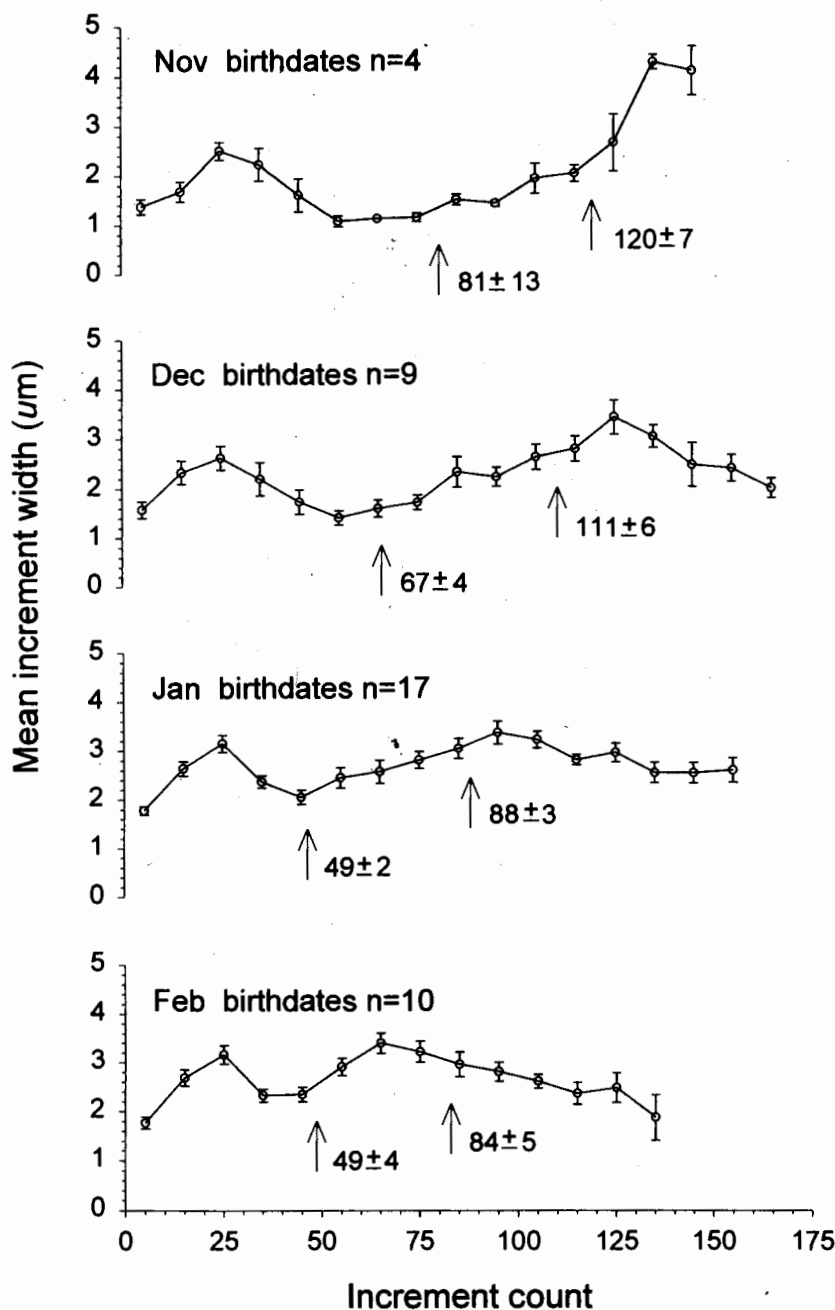


FIGURE 6.—Average increment widths ( $\pm$ SE) for each successive 10 increments from first feeding to the vertical edge of the sagittal cross sections for otoliths of randomly selected wild Atlantic menhaden grouped by month of birth. The initial increase and then decline in increment width corresponds to the at-sea larval stage, which occurred prior to capture. The arrows and associated numbers indicate the means ( $\pm$ SE) of the transition marks that correspond to changes in the axis of otolith growth observed in cross sections.

terns probably are detectable in the otolith record of Atlantic menhaden during the metamorphosis from the larval to juvenile stage. Although otolith features, such as metamorphic marks have been used to infer timing of metamorphosis, growth before and after metamorphosis, and duration of pe-

lagic larval stages of fishes (Francis et al. 1992; May and Jenkins 1992; Toole et al. 1993), we wished to determine if these features showed seasonal patterns that could be used to estimate birth dates.

Based on temperature-controlled rearing trials

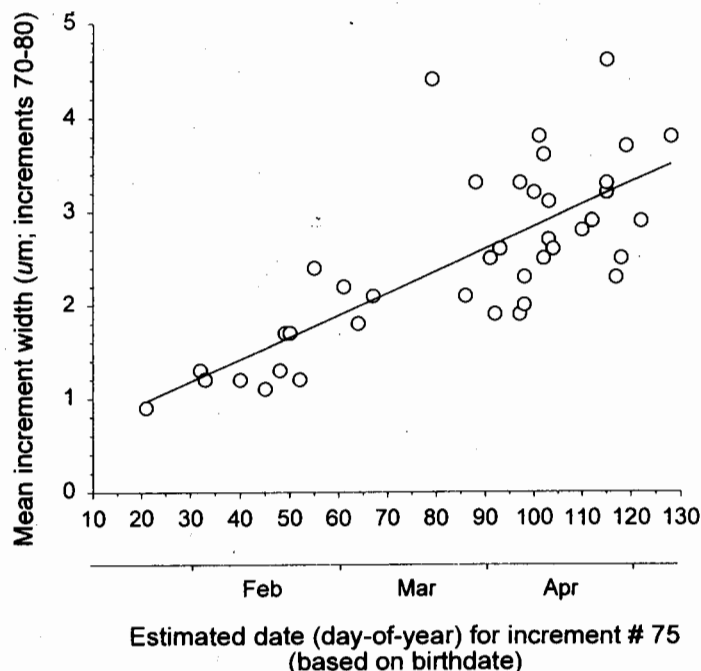


FIGURE 7.—Linear regression of mean increment widths between the zone of 70–80 increment counts on estimated date of deposition of increment 75 for randomly sampled wild juvenile Atlantic menhaden. Mean increment width =  $0.024 \times \text{day} + 0.47$ ;  $N = 40$ ,  $r^2 = 0.57$ .

TABLE 1.—List of variables and ranks used in the discriminant analysis. Count1 and count2 are increment counts to the first and second transition marks; M75 refers to the mean increment width between ring 70 and 80, etc. Hits are the number of correct classifications of wild Atlantic menhaden (out of  $N = 40$  observations) to month of birth for a single variable used to generate the discriminant function. Variables indicated in bold include the combination with the greatest hit rate (e.g.,  $26/40 = 65\%$  overall) and were used to generate the classification matrix.

Variable	Hits	Rank
<b>M75</b>	21	1.5
<b>M95</b>	21	1.5
<b>M65</b>	19	3.5
<b>Count2</b>	19	3.5
<b>M55</b>	17	5
Count1	15	6
M15	14	8
M85	14	8
M105	14	8
M35	10	11.5
M45	10	11.5
M115	10	11.5
M125	10	11.5
M5	7	14
M25	5	15

and observations from wild juveniles, interpretable records of water temperature were recorded within the otoliths of juvenile Atlantic menhaden. Water temperature signals were most evident from the position of otolith transition marks associated with metamorphosis and the increment spacing patterns within the zone of metamorphosis. The first and second transition marks occurred earlier as temperatures or spawning dates increased. The faster rate of somatic metamorphosis, visually observed with increasing temperature in rearing trials, was also evident from the otoliths. However, the transition marks were sometimes difficult to

TABLE 2.—Classification matrix for Atlantic menhaden whose birth months were independently determined by otolith aging and predicted based on the discriminant function.

Actual group	Number (%) classified as				Total
	Nov	Dec	Jan	Feb	
Nov	4 (100)	0 (0)	0 (0)	0 (0)	4
Dec	2 (22)	6 (67)	1 (11)	0 (0)	9
Jan	0 (0)	2 (12)	10 (59)	5 (29)	17
Feb	0 (0)	1 (10)	3 (30)	6 (60)	10

clearly distinguish. The mean increment count between the ALC mark and the second transition mark underestimated the number of days to metamorphosis for the ambient temperature group. This may indicate that the change in axis of otolith growth corresponding to the onset of the juvenile stage was less prominent and more difficult to detect in the ambient temperature treatment (change in the axis of otolith growth occurred more slowly at the cooler temperature) or that the narrowed increment spacing (often about 1  $\mu\text{m}$  or less near the first transition zone) led to an underestimate of total increments in this zone—a problem which was detected in earlier work (Ahrenholz et al. 1994). A more qualitative variable scoring the detectability of transition marks among individuals may have been useful to help interpret the temperature patterns, particularly for the discriminant analysis.

Increment width patterns, as opposed to increment counts, were more easily interpretable, and we noted a prominent increase in otolith increment widths with increasing temperatures. Mean widths increased from 1–2  $\mu\text{m}$  at 15°C to greater than 3  $\mu\text{m}$  at 25°C in a zone associated with metamorphosis. In wild-caught juveniles, this increase in increment spacing was most evident in the otolith after the zone where the first transition in the axis of growth occurs (about 70–80 increments).

An initial procedure for selection of discriminant variables also revealed that the increment widths in the zone associated with metamorphosis and the increment count to the second transition zone (onset of the juvenile stage) were the most predictive features for estimating birth months. In applying the discriminant analysis, fish were usually assigned to their correct month of birth, particularly for November and December, and in only one case, was a birth date misclassified by more than a month. Although this analysis has only been applied to juveniles from the 1993–1994 year-class, we expect that fall and midwinter birth dates can usually be discriminated because of the constancy of seasonal water temperature gradients off North Carolina (Stefansson et al. 1971).

The patterns we have described should be relatively common; about 85% of the Atlantic menhaden at Beaufort Inlet, North Carolina, colonize the nursery areas during rapidly warming temperatures (February to April), having been hatched from mid December to late January (Warlen 1994). We expect that an otolith pattern of early expanding increments followed by pronounced reduction of increment widths to be the most basic seasonal

pattern in the prejuvenile zone of the otolith. However, for individuals spawned very early or very late, other otolith features may be more diagnostic of birth date. For example, March birth dates are usually less numerous (annually <2% of the immigrating larvae at Beaufort Inlet; Warlen 1994), but preliminary information suggests that they would have unique and distinguishing otolith features. Relative to other North Carolina estuarine recruits, March-spawned Atlantic menhaden have the shortest at-sea duration and the smallest gradient of temperature change and experience the warmest temperature during metamorphosis (Warlen 1994). Transition rates would be faster and the early increment widths would probably not show the dramatic reduction we commonly observed for individuals ingressing in the winter.

We believe our approach to tracking birth dates among survivors shows promise, but if it is to be readily useful, some assumptions must hold. First, because the amount of otolith processing for increment measurements is time intensive and expensive, a single discriminant function should be constructed and refined. Observations from different years, such as the spring birth dates discussed earlier, should be added so that different functions need not be developed for each year-class. To justify the use of a single function, we have to assume that year-to-year temperature patterns are generally consistent and are recorded in the otoliths in a similar manner each year. Predicted birth dates from a single function could certainly be tested against ageable juveniles from multiple year-classes. Second, it is necessary to assume that geographic sources of recruits are consistent; that is, we are most concerned with tracking a temporal pattern of recruitment success (year-to-year differences among birth dates) rather than a geographical pattern. Results from a long-term juvenile abundance survey tentatively support this assumption of geographic consistency. Estimates of relative geographic contributions of recruits were obtained by extrapolation of the area of estuarine habitat by region and multiplying mean survey catch rates to determine stratified abundance means (Ahrenholz et al. 1989). About 80–85% of all recruits to the Atlantic fishery are derived from estuaries between Chesapeake Bay and Pamlico Sound. In the future, our samples may need to be tested for their degree of “representation” for this region.

Another assumption that must hold for the accurate interpretation of seasonal patterns in otoliths is that changes in increment widths within a

particular zone of the otolith are attributed to temperature and not other factors such as level of food—particularly starvation. In salmonids, temperature has been shown to have a more pronounced effect on otolith growth, via a proximal influence on metabolic rate, than somatic growth and food ration (Wright 1991; Bradford and Geen 1992). However, this relationship may not hold for other marine fishes, such as Atlantic menhaden, that have smaller eggs and larval sizes and that may be more susceptible to starvation. Starved Atlantic menhaden larvae do not exhibit systematic effects on increment periodicity but do exhibit decreases in otolith increment widths in comparison to well-fed control fish (Maillet and Checkley 1990). These reductions in increment width are as much as 1  $\mu\text{m}$  for larvae starved 1–3 d, indicating that short-term starvation events (a few days) may be detectable in the otoliths (Maillet and Checkley 1990). But we do not know if the variation in ration experienced by wild larval Atlantic menhaden could confound the interpretation of a temperature signal in otoliths. This uncertainty reinforces our belief that a discriminant analysis approach incorporating multiple otolith features, such as increment widths in different areas and variables related to metamorphosis, is necessary to distinguish temperature or seasonal effects from other environmental influences.

The value of our approach derives from the ability to track attributes of survivors from early life history to the adult stage. Daily age cannot be determined for adults and currently there are few alternative methods for characterizing survivors. For example, hatchery marking of larvae has been successfully used to track the fates of recruits, but often large-scale marking methods are constrained by costs or restrictions on chemical marking (Wilson 1995). Additionally, our approach offers the potential for monitoring wild populations, and the ability to discriminate less-abundant birth groups may be important in a long-term analysis of recruitment to the adult stock. Ahrenholz et. al. (1989) have shown a negative correlation between average length and year-class abundance at age of recruitment to the fishery that they think may indicate density-dependent nursery growth that is characteristic of large year-classes. However, other factors could contribute to this correlation, such as higher survival of the later-spawned individuals from late winter or spring birth cohorts. Our method should allow us to determine among-year relative birth cohort contributions using otolith transition zones, increment patterns, and postmeta-

morphic growth rates of year-classes surviving to enter the fishery. In addition, a comparison of birth dates within a year-class at several stages of ontogeny should indicate which, if any, stages are affected by selective mortality.

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